Supplemental Material to:
Armstrong CA, Jones GD, Anderson R, Iyer P, Narayanan D, Sandhu J, et al. DNMTs are required for delayed genome instability caused by radiation. Epigenetics 2012; 7(8);
http://dx.doi.org/10.4161/epi.21094
http://www.landesbioscience.com/journals/epigenetics/article/21094

Supplementary Table 1. Details of 6-TG colonies analysis

mESC line and treatment	Total number of 6-TG colonies	Colonies tested by exonic PCR	Number of unique mutations *	% Colonies screened
Wild type sham	5	5	4	80.00
Wild type 3Gy	32	10	8	25.00
Dnmt1-/- sham	127	16	15	11.81
Dnmt1-/- 3Gy	56	36	12	21.43
Dnmt3a-/- sham	4	2	2	50.00
Dnmt3a-/- 3Gy	4	4	3	75.00
Dnmt3b-/- sham	21	4	3	14.29
Dnmt3b-/- 3Gy	17	13	7	41.18
Dnmt3a3b --/-- sham	4	4	3	75.00
Dnmt3a3b --/-- 3Gy	9	6	6	66.67

* Mutations observed more than once in the same clonal population were counted only once

Supplementary Table 2 – Primers used to characterise mutations within the *Hprt* gene.*

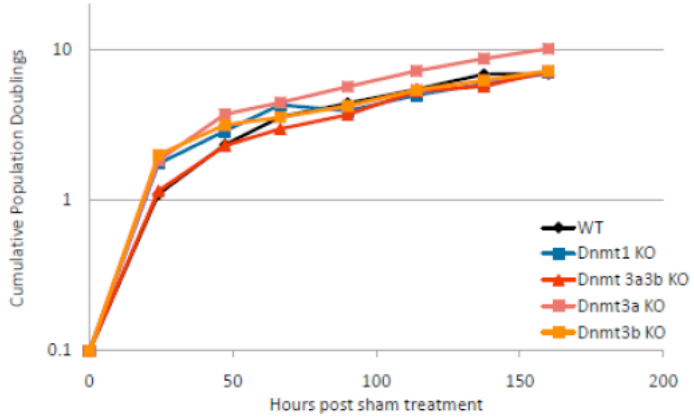
Primer Name	Sequence 5'-3' (taken from Meng *et al*, 2004).	Product size	Primer conc.	Alterations to basic conditions
Exon 1 F	ATCAGGCCCACCTAGTCAGA	320bp	1.5µl 50mM	67°C Tm No DMSO 11.1x buffer
Exon 1 R	CTCTGCTGGAGTCCCCTTG			
Exon 2 F	GCAGATTAGCGATGATGAACC	112bp	1µl 10mM	Touchdown
Exon 2 R	CCTGTCCATAATCAGTCCATGA			
Exon 3 F	CCTCATGCCCCAAAATCTTA	379bp	1.5µl 10mM	Touchdown No MgCl2
Exon 3 R	CACAGTAGCTCTTCAGTCTGATAAAA			
Exon 4 F	AGCATAATTTTGTGGCCATT	225bp	1.5µl 10mM	Touchdown No DMSO
Exon 4 R	AAAATGTTCTTTCTTTCCTCTTCAA			
Exon 5 F	TTTAAGGGCTCTGGTGGATG	552bp	0.75µl 10mM	Touchdown
Exon 5 R	CAACTCAGGCTAACCCAGGA			
Exon 6 F	TTAAGGCCACCAACCTGAAC	485bp	1µl 10mM	Touchdown No MgCl2
Exon 6 R	GGCATACATACCTTGCAACC			
Exon 7.8 F	CTGGTGAAAAGGACCTCTCG	262bp	0.4µl 10mM	Touchdown 1µl MgCl2 No DMSO
Exon 7.8 R	CAAGGGCATATCCAACAACA			
Exon 9 F	CCCAGACAACGTAGGAGGAC	196bp	1.5µl 10mM	Touchdown
Exon 9 R	TTACTAGGCAGATGGCCACA			
K-ras F	TTCTCAGGACTCCTACAGGAAA	191bp	1.5µl 10mM	55°C Tm No DMSO 11.1x buffer
K-ras R	CCCACCTATAATGGTGAATATC			

*11.1x buffer (0.5M TRIS pH8.8, 122mM Ammonium sulphate, 50mM $MgCl_2$, 75mM Beta-mercapto-ethanol, 0.05mM EDTA, 11mM dNTPs, 1.2mM BSA) was used instead of 10X buffer in the indicated PCRs. K-ras primers were used as control

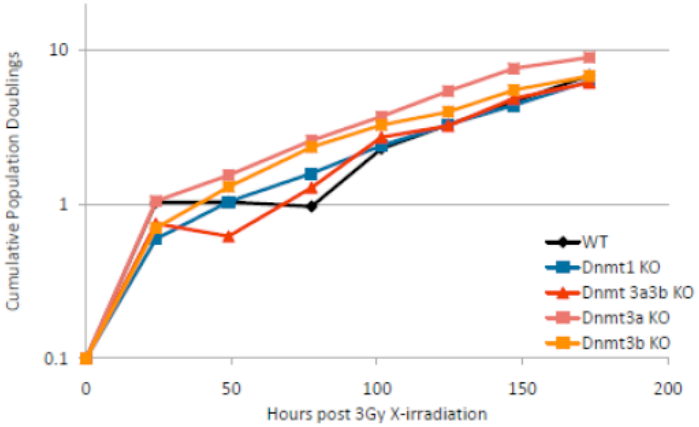
Supplementary Table 3 – Primers used to generate the LINE1 probe used for Southern blotting experiments in Supplementary Figure 5

Primer name	Primer sequence	Tm	Product size	Target sequence (murine)
LINE1 F	GCCTGCCCCAATCCAATC	63°C	206bp	5' monomer sequence of L1 A subfamily
LINE1 R	TGTGATCCACTCACCAGAGG			

A.



B.



Supplementary Figure 1 Growth rates, shown as cumulative population doublings, in unirradiated cells (A) and cells irradiated with 3Gy X-rays (B). Each point represents a single measurement. In agreement with published work,[27, 28] the *Dnmt* KO mESC lines showed no obvious abnormalities with respect to growth rate or morphology in comparison to the wild type cell line. Growth rates were similar between all five ESC

lines, both in untreated and 3Gy X-irradiated cells, although the *Dnmt3a*□/□ ESC line proliferated slightly faster than the other ESC lines. Cell proliferation was, however, slightly reduced during the first 3 days post treatment in X-irradiated cells compared to sham treated controls, possibly as a result of radiation-induced cell death or cell cycle arrest. The plateau in population doublings post irradiation was most apparent in the wild type and *Dnmt3a3b*□□/□□ cell lines, indicating that it occurs irrespective of global methylation levels. The growth curves appear to become slightly steeper at later time points (>72 hours) in the 3Gy X-irradiated cells, indicating a higher rate of cell proliferation compared to their unirradiated counterparts. This may be due to release from cell-cycle arrest and/or accelerated proliferation of surviving stem cells to recover the population. By the 7th day, both irradiated and unirradiated cell populations had undergone near identical numbers of population doublings.

The relationship between PDs and days in culture can be inferred from this figure: at the 5 day time point, unirradiated cells have undergone approximately 5-8 PDs, whilst 3Gy X-irradiated cells underwent 3-5PDs; at the 10 day time point, extrapolation of the graphs estimates that both irradiated and unirradiated cells would have undergone 9-14 PDs.

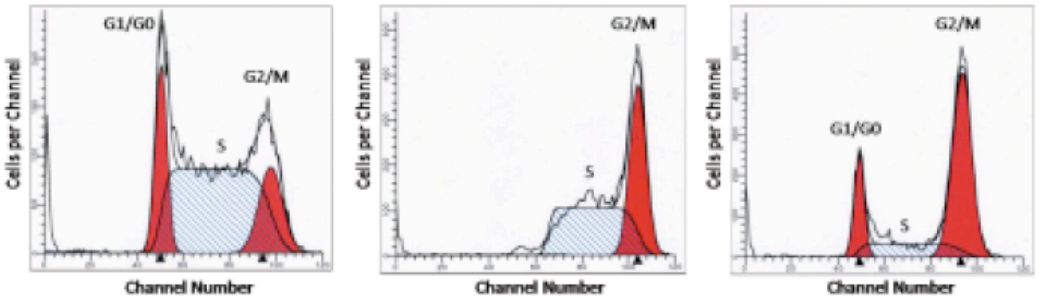
A

i.

ii.

iii.

C.

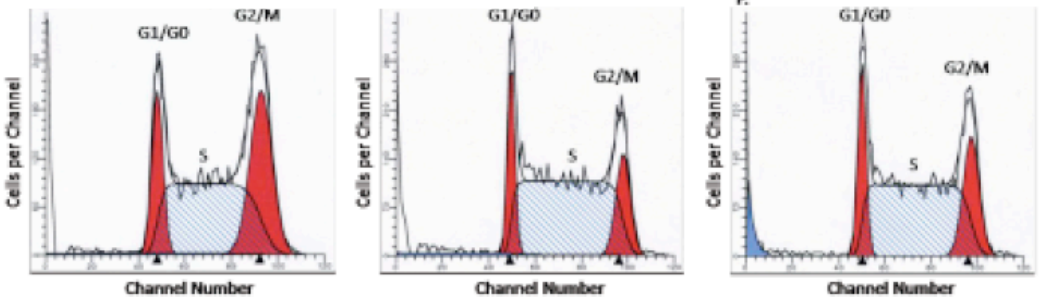


iv.

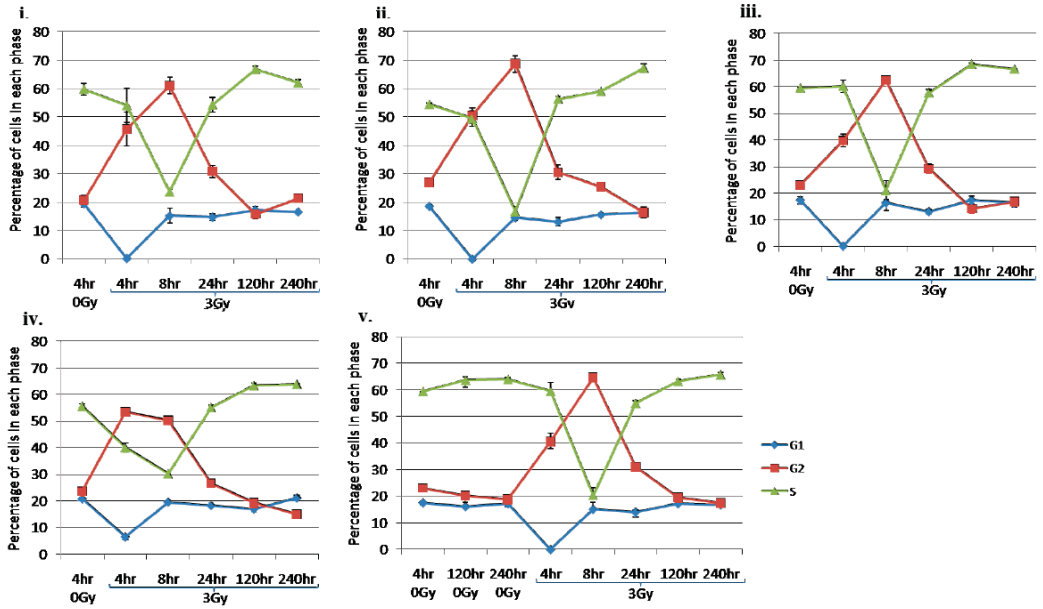
v.

vi.

F.



B



Supplementary Figure 2 (**A)** Representative histograms of wild type mESCs cell cycle distribution 4 hours post sham treatment (i.) and 4 hours (ii.), 8 hours (iii.), 24 hours (iv.), 120 hours/5 days (v.), 240 hours/10 days (vi.) post irradiation with 3Gy X-rays. As compared to somatic cells, mESCs have a reduced G1 phase and effectively absent G1 cell cycle checkpoint in response to ionizing radiation (Hong and Stambrook, 2004). This is evidenced at the 4 hour time point post irradiation by the progression of cells straight through G0/G1 and S phases to accumulate at the G2/M checkpoint. (**B**) Relative percentages of cells in each phase of the cell cycle at various time points post irradiation with 0 or 3Gy X-rays in wild type (i.), *Dnmt1-/-* (ii.), *Dnmt3a-/-* (iii.), *Dnmt3b-/-* (iv.), *Dnmt3a3b--/--* (v.) cells. Each data point represents the mean and error bars represent the SEM.

Hong Y, and Stambrook PJ. Restoration of an absent G1 arrest and protection from apoptosis in embryonic stem cells after ionizing radiation. Proc Natl Acad Sci U S A 2004; 101:14443-8

A

B

	Hprt Exons								Control
Sample	1	2	3	4	5	6	7-8	9	K-ras
Dnmt1-/- sham	N	N	N	N	P	N	P	N	P
	N	N	P	P	P	N	P	P	P
	N	N	N	N	P	N	P	N	P
Dnmt3a3b--/-- sham	N	P	P	P	N	N	N	N	P
Wild Type 3Gy	P	N	P	P	P	P	N	P	P
	P	P	P	N	P	P	N	P	P
Dnmt1-/- 3Gy	P	N	P	P	P	P	N	P	P
	P	N	P	P	P	P	N	P	P
Dnmt3a3b--/-- 3Gy	N	N	P	P	N	P	N	N	P
	N	P	P	N	N	P	N	N	P
Dnmt3a-/- 3Gy	N	N	N	N	P	P	P	N	P
Dnmt3b-/- 3Gy	N	P	N	P	P	P	P	P	P
	N	N	P	P	N	P	P	P	P
	P	P	N	N	N	N	P	N	P

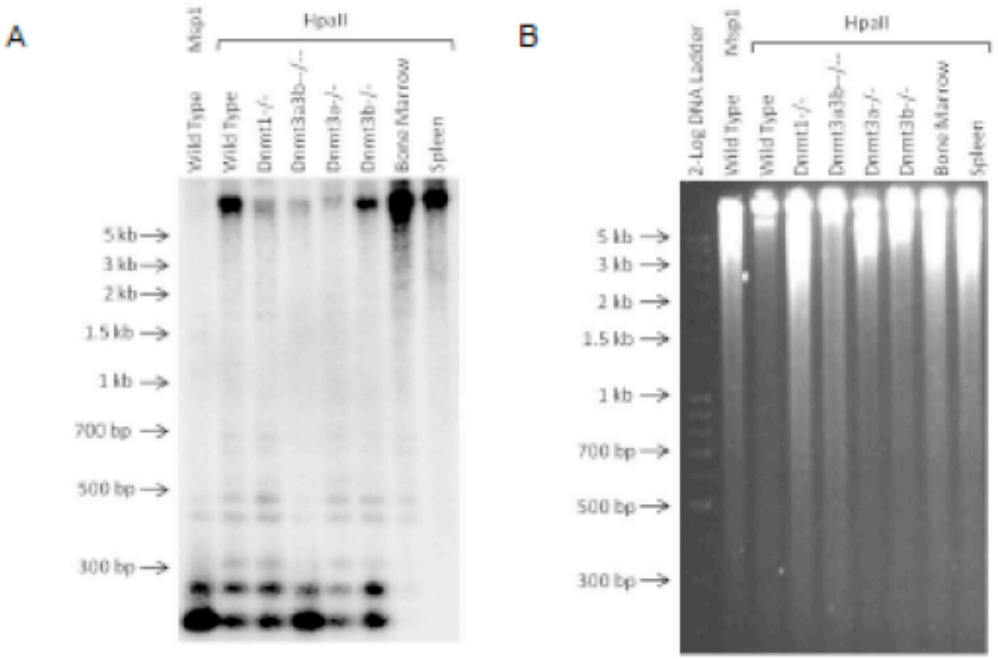
Supplementary Figure 3 Non-contiguous exonic deletions were observed in mESCs. (A) Schematic diagram the mouse *Hprt* gene showing the promoter region and all 9 exons. Scale is in kilo-bases (kb), with each minor unit 2kb. (B) Details of the non-contiguous exonic deletions observed in individual mESC lines. Exons for which a PCR product was present are identified by 'P'. Exons for which no PCR product was detected are denoted 'N'.

A

5′-***cctcatgccccaaaatctta***cctttggtatatgaaaaatagtct
ccacttctgcaaaatattgctttatgaagtaagaattcccttcatag
agacaaggaatgtgtcctgtaaaagtttaatgtgtaagaagtatttg
ttttttttttttttttttttttttttttttttttttttttttttttt
ttttaaatatttatttatttattatatgtaagtacactgtagctgtc
ctcagacactccagaagagggagtcagatcttgttatggatggtcgt
gagccaccatgtggttgctgggatttgaactcctgaccttcggaaga
gcagtcgggtgctcttacccactgagccatctcaccagcccc**agaag**
tatttgttataaaagataaatattcagaatcttctttttaattcctg
attttatttctatagGACTGAAAGACTTGCTCGAGATGTCATGAAGG
AGATGGGAGGCCATCACATTGTGGCCCTCTGTGTGCTCAAGGGGGGC
TATAAGTTCTTTGCTGACCTGCTGGATTACATTAAAGCACTGAATAG
AAATAGTGATAGATCCATTCCTATGACTGTAGA***TTTTATCAGACTGA***
AGAGCTACTGTgtaagtataattaacttataattaaaaaaatagggc
c-3′

B

Supplementary Figure 4 SINE insertions in Dnmt1-/- cells (A) DNA sequence of *Hprt* gene exon 3 and surrounding introns, and the B2 SINE insert. (B) DNA sequence of *Hprt* gene exon 6 and surrounding introns, and the B1 SINE/Alu insert. Bold Italic shaded = primers; lower case = intron; upper case = exon; black underlined = SINE insert; grey shading = duplicated DNA sequence adjacent to the insert.



Supplementary Figure 5 Genomic DNA from mESCs and mouse spleen and bone marrow was digested with *Msp*1 or *Hpa*II and analysed by Southern blot with probe specific LINE1s (A), A 2-Log DNA ladder (NORGEN) was used as a size marker, and the ethidium bromide stained gel image, pictured 16 hours after loading the samples, was used as a loading control (B). The Dnmt3a3b--/-- ESCs were at passage 22.